Exploration of Cochlear Potentials in Guinea Pig with a Microelectrode*

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(Received May 13, 1954)

The ac ("microphonic") cochlear potential and the positive dc "endolymphatic" potential have been recorded simultaneously as the exploring electrode was introduced into scala media or as other parameters were varied. Negative intracellular dc potentials were demonstrated in the cells of the organ of Corti. The zone of positive endolymphatic potential is bounded by the reticular lamina, not by the basilar membrane. The cochlear microphonic reverses phase as the exploring electrode penetrates the reticular lamina. A dc polarizing current with the positive pole in scala media (and negative in scala tympani) increases the cochlear microphonic just as it does when the positive pole is located in the scala vestibuli. These facts indicate that the source of the ac (microphonic) potential seems clearly to be at the hair-bearing end of the hair cells and that the source of the dc endolymphatic potential is probably here also, while Reissner's membrane is not the source of either the ac or the dc potential. No steady dc current flow outside scala media was found such as would be expected if stria vascularis were the dc source and if the hair cells modulated a dc current flow through them. The dc endolymphatic potential may be increased by as much as 10 percent if and while the basilar membrane is displaced toward scala vestibuli and may be decreased to 50 percent or less when and while it is displaced toward scala tympani. Isotonic solutions rich in potassium depressed the ac potential and nerve responses when introduced into scala tympani but not when in scala vestibuli only. The dc potential, however, was not altered by high potassium concentration in scala tympani.

1. INTRODUCTION

WHEN sound enters the ear, the cochlea develops two electric responses. One is the "cochlear microphonic" (CM) and the other is the nerve action potential. There are also resting dc potential differences between different parts of the cochlea[1–4]. The purpose of this paper is to describe further the relationship between the dc and the microphonic potentials in the cochlea and to explore the possible sources of these potentials. To a large extent our observations and conclusions are repetions of those of Békésy. We are almost entirely in agreement with him so far as our observations are comparable.

The dc potentials in the cochlea apparently have two different origins; one is the internal resting (or injury) potential of the cellular elements in the cochlea, and the other is an extracellular potential difference between the endolymph and the perilymph. The latter we shall call "the dc endolymphatic potential." Using a submicroscopic glass-pipette electrode we have recorded the ac microphonic and both dc potentials in various parts of the cochlea under varying experimental conditions. Although insertion of a microelectrode into a hair cell seems to injure a cell seriously, we have been able to record both dc and ac potentials from the region of the organ of Corti in spite of the vibratory motion caused by sound.

2. METHODS

Guinea pigs under dial anaesthesia were used. The techniques of opening the bulla, of drilling small holes, of inserting nonpolarizable or wire electrodes in various turns of the cochlea and of fixing these electrodes to the edge of the bulla have been described elsewhere.[5,6] The head of the animal was fixed to the table with three small clamps, one holding the incisor teeth, the second one fixing the zygomatic arch and the last one catching the edge of the bulla. The tympanic membrane was left intact.

Sound stimuli used in these experiments were either tone pips[7] or pure tones. The sound was applied through the opening in the bulla with the external auditory meatus of the animal blocked with a piece of plasticene.

Microelectrodes were pulled by hand from soft or Pyrex glass tubing of approximately 1-mm diameter. A 3-molar KCl solution was introduced by boiling electrodes in alcohol and then replacing the alcohol with the KCl solution.[8] The microelectrode was held by a small clamp made of a plastic rod and was slowly pushed by a micromanipulator into scala media through a small hole made in the bony wall over the stria vascularis or else through a large opening in scala tympani of the basal turn.

Cochlear potentials were recorded by two or three independent cathode-ray oscillographs with either dc or condenser-coupled amplifiers. For microphonic and nerve responses, nichrome wire electrodes and

* This work was supported by a contract between the U. S. Office of Naval Research and Central Institute for the Deaf.

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[2] G. von Békésy, J. Acoust. Soc. Am. 24, 72–76 (1952).

[3] G. von Békésy, J. Acoust. Soc. Am. 24, 399–409 (1952).

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[6] Tasaki, Davis, and Legouix, J. Acoust. Soc. Am. 24, 502-519 (1952).

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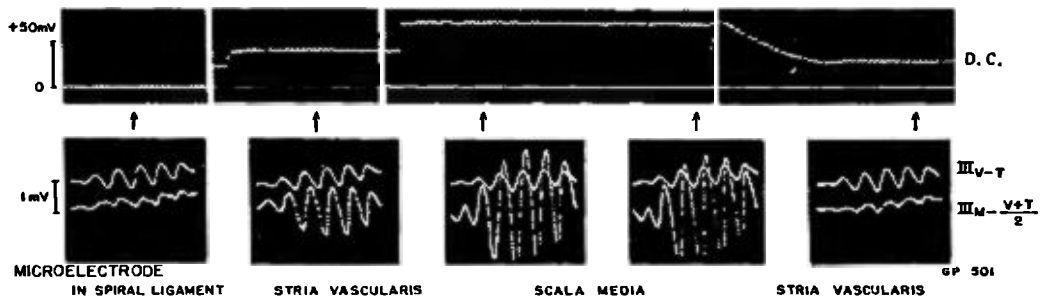

FIG. 1. Penetration of a microelectrode into scala media of third turn III through stria vascularis. The uppermost trace shows the potential difference between the microelectrode and the reference electrode in scala tympani of the basal turn. The 60-cps ripple shows the speed of the film. The third trace shows the microphonic responses to 500-cps tone pips from the third turn (vestibuli *V*, to tympani *T*) taken at high transit speed at the moments indicated by the arrows. The lowest records show the microphonic responses taken through the microelectrode *M* simultaneously with the middle records, and referred to the average potential of *V* and *T*. The estimated position of the microelectrode is given beneath.

the cathode-follower preamplifiers described in a previous paper were used. By adjusting the potentiometers connected to the outputs of these preamplifiers we could separate the local microphonic responses from the nerve responses and other electric potentials of remote origin.[6] When the dc cochlear potential was recorded through a microelectrode, the grid of the cathode-follower was connected by a fine silver wire directly to the fluid in the microelectrode. No blocking condenser nor grid resistance was in the grid circuit. However, the electrode resistance was very high (20–30 *M*), and the grid current was correspondingly low.

3. DISTRIBUTION OF MICROPHONIC AND DC POTENTIALS IN THE COCHLEA

A pair of nichrome-steel wire electrodes were inserted on opposite sides of the scala media, one in scala vestibuli and the other in scala tympani, of either the third or the basal turn to record the cochlear microphonic (CM) responses. A microelectrode about 1μ in diameter at the tip was inserted into the scala media through the stria vascularis. As reference electrode a large glass-pipette electrode (approximately 100μ inside-diameter at the tip) was inserted in the scala tympani of the basal turn. The lower half of this electrode was filled with ringer-agar gel and its upper half with a 3-molar KCl solution. Weak low-frequency tone pips (usually 500 cps) were delivered at the rate of 3 to 7 per sec.

When the microelectrode was in contact with the fluid in the spiral ligament just outside the stria vascularis (Fig. 1, left) the dc potential at the tip of the microelectrode was never more than a few millivolts. At this same position the microphonic response (referred to the average of the potentials in scalae vestibuli and tympani) was also very small. In Fig. 1 this channel (lowest trace) shows nothing but action potentials of the nerve.

When the microelectrode was pushed into the stria vascularis, the dc channel showed irregular variations of potential. The potential in the stria was often positive to the potential of the perilymph. When a positive potential appeared, there was always an increase in the microphonic response picked up by the same microelectrode. The microphonic response recorded from the pair of metal electrodes in scalae vestibuli and tympani was not affected by inserting the microelectrode into the stria.

When the microelectrode was pushed farther inward there suddenly appeared a large positive potential, generally 60 to 90 mv in magnitude, which remained unchanged as long as the electrode stayed in that position. It was not very easy to identify the exact location of the microelectrode when the large positivity appeared, because of the high elasticity of the spiral ligament and the stria vascularis. In the third turn the positivity appeared when the tip of the microelectrode had advanced 0.3 to 0.4 mm inward from the surface of the spiral ligament. When the microelectrode was slowly withdrawn the potential disappeared at about 0.2 mm from the surface. Frequently the disappearance was not as abrupt as its appearance during the forward movement of the microelectrode (Fig. 1, right top). This hysteresis indicates a dimpling of the tissue before penetration and some backlash on withdrawal.

The large positive potential regularly remained constant in spite of further large forward movements of the electrode. The potential was found with much larger (20μ) electrodes as well as with small ones (1μ or less). It seems clear that this positivity is the dc potential of the endolymph described by Békésy and not an intracellular potential of any of the cells surrounding the scala media.

The appearance of the large positive dc potential at the tip of the microelectrode was always accompanied by a great increase in the amplitude of the microphonic response. The ratio of the amplitude of the microphonic response (to tone frequencies between 100 and 250 cps) recorded from scala media to the microphonic measured between scala vestibuli and scala tympani

of the same turn was approximately 6:1 in the third turn and 5:2 in the basal turn (see also Davis, Tasaki, and Goldstein[4]).

In Fig. 1 there is a slight phase difference between the two microphonic responses. The vestibuli-to-tympani differential response precedes the endolymph-to-average wave. This phase difference is small, but it was present in all records from the third turn with either pips or tones and to a smaller extent in records from the basal turn. Békesy has already described this phase difference.[3]

Figure 2 is an example of the results obtained by pushing the microelectrode through the basilar membrane of the basal turn. The thick line on the right-hand side of the figure records the potential difference between the exploring microelectrode and the fixed glass-pipette electrode in the scala vestibuli of the basal turn. As the microelectrode was slowly advanced through the basilar membrane, there appeared first small, somewhat unsteady, potential variations which were sometimes positive (as in this example) and sometimes negative relative to the reference electrode. Then, in almost all of our experiments, a large *negative* potential, sometimes greater than 50 mv (as in the second strip in Fig. 2) was observed for a short period in the cellular layer above the basilar membrane. A further advance of the microelectrode always resulted in the sudden appearance of a large *positive* dc potential (the third strip in Fig. 2). All of these observations are in good agreement with Békésy's descriptions,[1–3] although he does not describe in detail his explorations in the neighborhood of the hair cells.

During the penetration of the microelectrode through the region of the organ of Corti there are dramatic changes in the size and the phase of the microphonic. In Fig. 2a weak pure tone of 500 cps was delivered to the animal. The left channel of the oscillograph shows the microphonic potential between the scala vestibuli and the microelectrode. When the tip of the microelectrode was in the scala tympani the usual small vestibuli-to-tympani microphonic was observed (Fig. 2 top). When the microelectrode was pushed deeper into the region of the organ of Corti there was frequently a very pronounced increase in its amplitude (second strip in Fig. 2).

When the microelectrode finally reached the region of strong positive potential there was always an abrupt reversal in the phase of the microphonic (the third strip in Fig. 2). Then, as long as the positive dc was maintained, further advancement of the microelectrode into the scala media did not cause any noticeable change in the amplitude or phase of the microphonic wave.‡

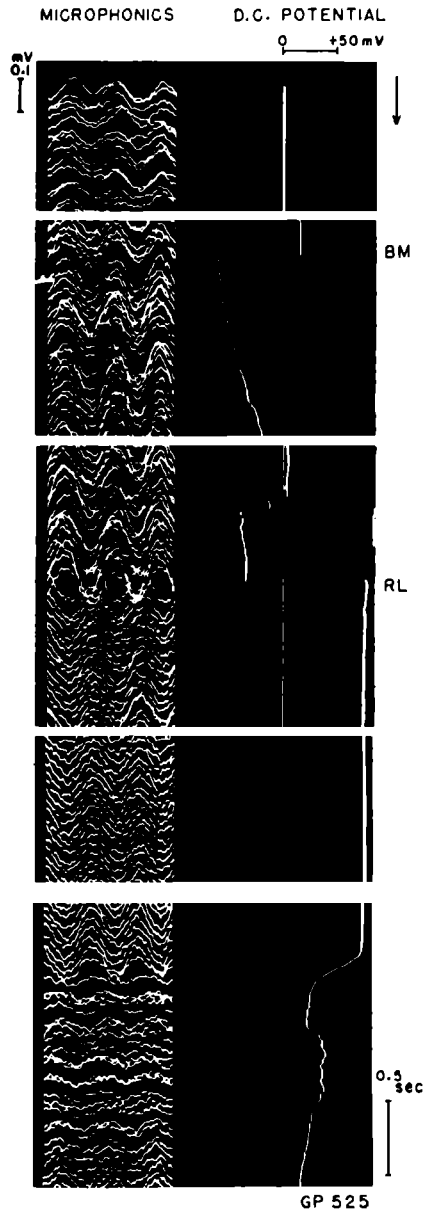

FIG. 2. Penetration of a microelectrode into scala media of the basal turn through the basilar membrane. The dc potential and the microphonic responses to a 500-cps tone were recorded between the microelectrode and a reference electrode in scala vestibuli. The dotted part of the dc line is only approximate as the beam went slightly off the screen at this time. Only a part of each sweep for the microphonic was photographed. *BM* indicates the time at which the microelectrode started to record a large negative potential and *RL* the moment at which the reversal in the phase of the microphonic response and also in the dc potential took place. In the lowest section the electrode was withdrawn. The regularity of the microphonic just before withdrawal shows that the dc potential was steady.

In some trials we saw a clear *decrease* in the amplitude of the microphonic wave in a region close above the basilar membrane. These cells seem to be Békésy's "cells with a wall."[3] It was our strong impression that such decreases in amplitude of the microphonic wave occurred when the penetration was made near the

‡ In a previous paper (see reference 4) we (HD and IT) were in error in drawing an equipotential contour (for the microphonic) *within* the scala media. In Fig. 3 of reference 3 the equipotential line should follow the boundary of the endolymphatic space, i.e., the heavy line in Fig. 3 of the present paper.

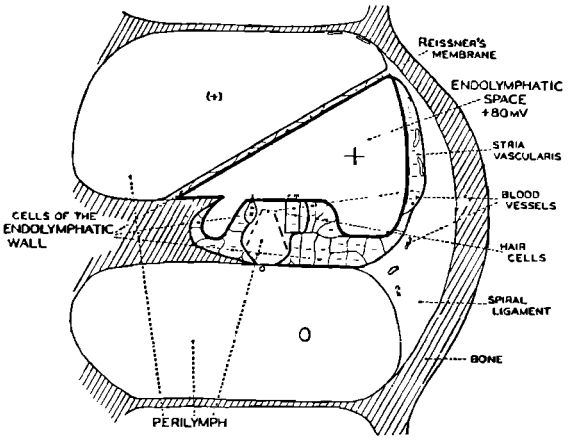

FIG. 3. Interpretive diagram of the endolymphatic space and the distribution of dc potentials within the cochlea. The heavy line represents the boundary of the endolymphatic space. The potential within it is uniform and strongly positive relative to scala tympani. The walls of the endolymphatic space are composed of cells. Within these cells, while uninjured, the potential probably averages about −40 mv relative to scala tympani. Our measurements run as high as −60 mv in the organ of Corti. For Reissner's membrane Békésy says (see reference 2) "−20 mv and may have been even higher." The degree and distribution of the negativity in stria vascularis is still very uncertain. The negative intracellular potential rapidly decays or is lost if the cell is seriously injured by the exploring electrode. The tectorial membrane seems to be electrically "transparent," like the spiral ligament, and is omitted in this diagram. Only one external hair cell is shown. The reasons for indicating perilymph in the tunnel of Corti are given in the text.

external edge of the basilar membrane, namely in the region of the cells of Hensen and Claudius, but, because of optical difficulties of observation due to gradual accumulation of perilymph on the surface of the basilar membrane, we could not make accurate measurements of the distance between the point of penetration and the edge of the spiral lamina.

So far the chief difference between our observations and those of Békésy is our frequent finding of positive potentials in the stria vascularis. Békésy states that the potential here was "always negative." His negative potentials are apparently intracellular in origin. Békésy's electrodes were more carefully aligned than ours and were introduced parallel to the axis of the modiolus, apparently with little stretching or injury of the tissue. Our positive potentials in the stria, and occasionally also in the organ of Corti, could be due to injury in the neighborhood of the electrode and a resulting leakage of positivity from the scala media.

Our best present picture of the distribution of the dc potentials, based on our observations and those of Békésy are given in Fig. 3. The tectorial membrane, which is noncellular and colloidal in structure, is omitted in the diagram. No potential differences that might be ascribed to it have been found. The small positive potential in the scala vestibuli, described by Békésy,[1] is shown by a small plus sign in parentheses. The potential of the fluid in scala tympani is zero by definition.

The negative dc potentials above the basilar membrane are undoubtedly of cellular origin. It is apparently the familiar negative potential that is found inside most, if not all, living cells and which is the basis of the "injury potential" of a partially damaged nerve or muscle fiber. The potential of the stria vascularis is shown as negative as though it were always measured *inside normal* cells. We accept Békésy's observation of negative intracellular potentials within the cells of Reissner's membrane and have not attempted to confirm them. The spiral ligament is practically equipotential with scala tympani throughout.

The very similar distribution of the cochlear microphonic is given in Fig. 6 in comparison with a simplified version of the distribution of the dc potentials.

4. VARIATION IN POTENTIAL OF ENDOLYMPH CAUSED BY PRESSURE IN SCALA VESTIBULI, TYMPANI, OR MEDIA

The effect of a difference in hydrostatic pressure between scala vestibuli and scala tympani on the dc cochlear potential was investigated, particularly to determine the polarity of the resulting electrical change.

A pair of large holes was drilled in the basal turn, one in the scala vestibuli and the other in scala tympani. A reference electrode (Ag-AgCl-3-molar KCl-ringer agar-gel) was inserted through another hole into scala tympani of the basal turn. A small hole, approximately 50μ in diameter, was made through the bony wall outside the stria vascularis of the third turn and a microelectrode was pushed through it into scala media to record the dc potential. The nerve responses to clicks or to tone pips were observed from time to time and those preparations which did not give good neural responses were discarded.

A small amount of mammalian ringer solution was now blown into the perilymphatic space from a small

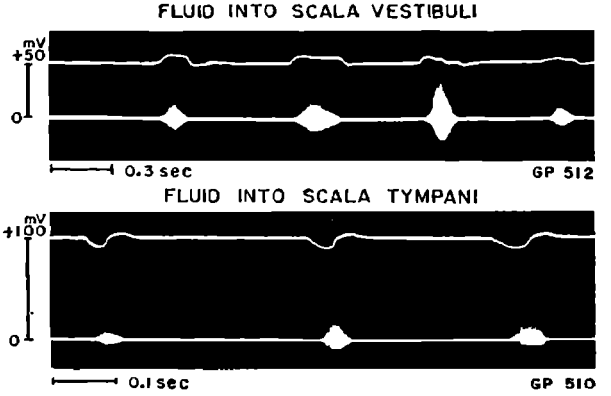

FIG. 4. Effect of pressure applied to the cochlear partition upon the dc potential in the scala media. In the upper record mammalian ringer fluid was forced into scala vestibuli of the basal turn and allowed to flow out of a hole in scala tympani of the basal turn. In the lower record the fluid ran in the reverse direction. The broadening of the zero line shows the relative intensity of flow of the fluid. The nonlinearity of the electrical change is obvious.

glass pipette which was held by hand and closed the hole as tightly as possible during the injection. The fluid was allowed to run out of the other hole freely, causing a steady flow through the helicotrema. Both the stapes foot plate and the round window always moved outward during the injection. The rubber tubing connected to the pipette was provided with a side tube which had a small hole at its end. The hissing sound of escaping air, picked up by a nearby microphone, signalled the start and the relative intensity of compression of the air in the tubing.

Figure 4 shows how the positivity in the scala media was increased by nearly 10 mv when positive pressure was applied to *scala vestibuli*. The increase in the positive potential was apparently limited by some intrinsic mechanism in the cochlea. A plateau was reached although the pressure continued to increase. This plateau was in some preparations considerably less than 10 mv, but in such cases a large transient decrease in the dc potential was generally observed on withdrawal of the pressure. This suggests that an accommodation of some sort to the increased pressure had occurred.

On the other hand, positive pressure in the *scala tympani* always caused a distinct reduction in the dc potential. It fell to less than half of its original value when the applied pressure was very strong. Such a fall is much greater than the greatest increase we ever observed with the opposite pressure. When the inflow of the fluid into the scala tympani came to an end there was always a transient elevation in the potential of the endolymph above its resting level. We shall not attempt to explain in detail the irregularities, the overshoots and the nonlinearity of response we have observed. The recent studies by Békésy[9,10] should be consulted for the best detailed information in this area.

In another set of experiments pressure differences across the cochlear partition were produced by variations in the air pressure in the external auditory canal. The cochlea had no open holes in it in this case. The dc potential was recorded from a microelectrode in scala media of the third turn as usual. Air was blown into or sucked from a rubber tube connected to the ear canal. Increase in air pressure usually caused a transient increase in the positive dc potential and reduction in pressure a decrease. There was a marked tendency to a positive overshoot after suction was ended and sometimes a brief increase preceded the usual fall when quick suction was attempted.

The hydrostatic pressure *within the scala media* was changed by means of a glass pipette, 20 to 30μ in diameter, that was pushed slowly into the scala media, second turn. The pipette was filled with an isotonic potassium chloride solution because chemical analysis[11] has shown that the chief cation in endolymph is not sodium but potassium. A chlorinated silver wire was inserted in the fluid. A reference electrode was placed on the tissues of the neck. When the usual positive dc potential appeared on the screen of the oscilloscope a droplet of the isotonic KCl solution with a volume of about 0.015 mm^3 or less was injected into the scala media by blowing through a rubber tube as before. Alternatively endolymph was sucked out of scala media instead.

An injection into the scala media caused an immediate increase in the dc potential. This increase was sometimes about 5 mv and sometimes as much as 20 to 25 mv. When the blowing of fluid ceased there was always a gradual return of the dc potential toward the original level. Suction applied through the glass pipette caused a reduction in the dc potential, usually 20 to 30 mv depending on the intensity of the suction. Recovery took place gradually in 10 to 15 seconds after the end of suction. The slow recoveries may represent gradual equalizations of pressure by slow movement of fluid into or out of scala media.

The result of this experiment is complicated by streaming potentials but our tests so far show that the latter are not larger than 3 to 4 mv under the conditions of our experiment. Ringer solution gave the same immediate result as isotonic potassium chloride solution but seemed to cause a slow subsequent deterioration of the dc potential.

In summary, three different applications of pressure cause a reduction in the positive dc potential of the endolymph, (1) negative pressure applied to scala vestibuli (with scala tympani exposed to atmospheric pressure), (2) positive pressure applied to scala tympani (with scala vestibuli exposed to atmospheric pressure), and (3) negative pressure in scala media. The opposite pressures all cause definite, although smaller, increases in the dc potential.

In the displacements caused by the three maneuvers that reduce the potential the constant feature is the "upward" movement of the basilar membrane toward scala vestibuli, and movement of the basilar membrane toward the scala tympani is correlated with an increase in the potential. The change of potential in our experiments was quite well maintained as long as the pressure was maintained. *The magnitude of the dc endolymphatic potential is thus clearly correlated with the displacement of the basilar membrane.* Reissner's membrane is conclusively eliminated as source of the changes in dc; and it is difficult to imagine that the stria vascularis, if it were the source of the dc, would vary its output so markedly in response to mere changes of pressure. (The stria vascularis is not *displaced* by the pressure as is the basilar membrane.) We conclude that the slow modulations of the dc potential are caused by some structure that lies on the basilar membrane. By inference the actual source of the endolymphatic potential lies on and moves with the basilar membrane (see Sec. 6).

[9] G. von Békésy, J. Acoust. Soc. Am. **23**, 29–35 (1951).
[10] G. von Békésy, J. Acoust. Soc. Am. **25**, 786–790 (1953).
[11] Smith, Wu, and Lowry, Science **116**, 529 (1952).

5. THE EFFECT OF A DC POLARIZING CURRENT ON THE COCHELAR MICROPHONICS

In a previous study of the effect of electrical polarization of the cochlear partition on the cochlear microphonic response[5] the current was passed across the entire partition from scala vestibuli to scala tympani. We have now sent a polarizing current directly into scala media. Two nichrome-steel wire electrodes were placed in scala vestibuli and scala tympani respectively of the third turn for the recording of the cochlear microphonic. The current was introduced through two glass pipettes approximately 25μ in outside diameter and filled with mammalian ringer solution. One pipette was inserted into scala tympani of the basal turn, the other into scala media of the third turn. These polarizing electrodes were connected to the dc recording channel while one of them was being introduced through the stria vascularis of the third turn. The appearance of the usual large positive dc potential indicated when this pipette had successfully penetrated into the scala media. Then the two pipette electrodes were switched from the dc recording circuit to a polarizing circuit, which consisted simply of a battery of 250 v, a dc microammeter, a current reverser and a switch. To avoid formation of bubbles,[12] the current was not allowed to flow longer than about 5 sec.

When the polarizing electrode in the scala media was connected to the source (positive) of the direct current, there was an increase in the amplitude of the microphonic response, as shown in Fig. 5. A current flowing in the reverse direction diminished the response. These changes were reversible. As in the previous experiments,[5] there was a slight overshoot on withdrawal of the current, so that the amplitude of the microphonic was slightly subnormal for the first one to two seconds after interrupting a current which had made the scala media more positive and had increased the cochlear microphonic, and vice versa.

In the present and in the previous experiments the direction of current across the organ of Corti that caused increase of the microphonic was the same, i.e., toward scala tympani. The direction of current across Reissner's membrane, however, was opposite, since the source in one case was in scala media, in the other in scala vestibuli. We conclude, therefore (with Békésy[3]), that Reissner's membrane is not the source of the cochlear microphonic.

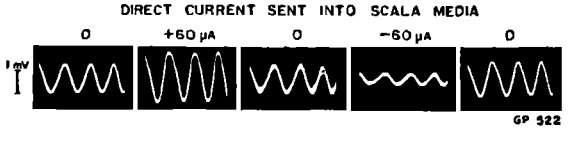

FIG. 5. Effect of direct current sent into the scala media upon the microphonic response to a 400-cps tone. The microphonic response was recorded from the perilymph in scala vestibuli and scala tympani opposite the spot at which the direct current was introduced.

[12] Fernández, Gernandt, Davis, and McAuliffe, Proc. Soc. Exptl. Biol. Med. 75, 452-455 (1950).

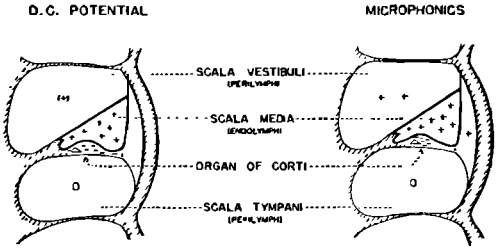

FIG. 6. Comparison of distribution of dc and microphonic potentials. The heavy line shows the boundary of the endolymphatic space (see Fig. 3). The tectorial membrane, stria vascularis and other details are omitted. The scala tympani is taken as arbitrary reference (zero) for both potentials. The number of symbols either + or − in an area indicates the relative magnitude of the potential. The negative dc potentials in the organ of Corti are intracellular potentials. The "negative" microphonic potentials in the region of the hair cells are microphonics that are opposite in phase to those in scala media. The microphonics in scala vestibuli and in the spiral ligament are relatively larger than the dc potentials there, presumably because of the low electrical impedance offered by Reissner's membrane to ac and its high impedance to dc.

6. THE LOCATION OF THE SOURCE OF MICROPHONIC AND DC POTENTIALS

The distribution of the dc endolymphatic potential was summarized in Fig. 3, and the distribution of the cochlear microphonic looks very similar (Fig. 6). If scala tympani is taken as the reference point the largest microphonic (ac) voltage is found when the exploring electrode is in scala media. The voltage is less, but still in nearly the same phase, in scala vestibuli and still less in the spiral ligament. The microphonic is strong in the stria vascularis, particularly when the exploring electrode shows a positive (extracellular) potential here, and it is nearly in phase with scala media. It is also very strong in the region of the hair cells, but here it is opposite in phase to that in scala media regardless of whether the dc potential is strongly negative (inside a hair cell) or near zero (extracellular).

The path of ac current flow seems clear, i.e., from the scala media across Reissner's membrane (mostly by capacitative effects) to scala vestibuli and thence by way of the spiral ligament, and presumably by other less direct paths also, to scala tympani. There may also be significant capacitative current flow across the stria vascularis into the spiral ligament. The return to scala media is clearly through the region of the hair cells where large ac potentials in opposite phase to scala media are found. The dramatic change of phase when the electrode penetrates the hair-bearing end of the hair cells into scala media locates the source of the ac (microphonic) potential at this boundary.

We believe that the conclusion that the source of the (ac) cochlear microphonic is at the hair-bearing end of the hair cells and oriented perpendicular to the reticular

lamina is consistent with all of our observations and also with those reported by Békésy and by others.

The source of the dc endolymphatic potential can also be located as somewhere in the wall of the scala media, because it is in the endolymphatic space within the scala media that the dc potential is highest. The search for the sink, i.e., the lowest dc potential, however, is complicated by the presence of the intracellular potentials. We therefore tried to find how, where, and how much the dc can be modified. The effect of large slow changes in pressure was found to be clearly related to displacement of the basilar membrane (see Sec. 4), and the inference is almost inescapable that these slow pressure-induced changes represent an intermediate link between the resting dc potential and the (faster) cochlear microphonic. A single source of potential that can be modulated by mechanical displacement (or deformation) is postulated. The modulation certainly occurs on the basilar membrane and almost certainly in the hair cells. The simplest assumption is that the source of the dc potential is also located in the hair cells and that it is modulated at its source by mechanical displacement. This does not imply that the same physicochemical mechanisms are necessarily involved both in generation and modulation but simply that if there is more than one mechanism they are nevertheless located in the same cells.

An alternative hypothesis was suggested by one of us,[13–15] namely, that the source might be located in the stria vascularis and that only the modulation, thought to depend on a change in ohmic resistance, occurs in the hair cell. The ac changes in potential were attributed to changes in the *IR* drop in a resting current that was assumed to flow from stria vascularis across scala media, through the hair cells (and nerve fibers), across the basilar membrane to scala tympani, and thence, by way of spiral ligament or blood stream, back to stria vascularis. An essential feature of this hypothesis is a flow of current *outside* the scala media from one point on the wall (under the tunnel of Corti) to another, i.e., the stria vascularis.

We have tested this hypothesis by seeking for evidence for such dc current flow. We reason that if such a current normally crosses the scala tympani the flow should be greatly altered by removing the perilymph, and that the alterations would be revealed by changes in dc potential between points in the path of the current outside scala media. Four such tests were made, all with negative results, with electrode placements as follows:

(1) Scala vestibuli and scala tympani of Turn I.

(2) Two positions in scala tympani, one in a small niche drilled into the spiral lamina and the other at the junction of the basilar membrane with the spiral ligament.

(3) Two positions in the spiral ligament, one near the attachment of Reissner's membrane and one near scala tympani.

(4) On the basilar membrane under the tunnel of Corti in the basal part of Turn I and another position in the more apical part of Turn I.

Only in the last case did the dc potential between the two electrodes change by more than 1 mv when perilymph was sucked out of tympani in the basal turn or when it was replaced again. A small change (3 mv) in the last case indicated a small current normally flowing longitudinally (basalward) in scala tympani. Such a current flow was inferred by Békésy[1] when he found a dc potential gradient along the cochlea.

In a rather similar test the dc potential between a point behind stria vascularis and scala tympani was found to remain constant while large reductions (50 percent) or increases (10 percent) in the endolymphatic potential were produced by slow changes of pressure in scala tympani.

These negative results show that any dc currents outside the scala media are extremely small, and seem inadequate to account, on the resistance-modulation theory, for the large changes in dc that can be produced by changes in hydrostatic pressure.

7. EFFECT OF POTASSIUM CHLORIDE ON THE COCHLEAR MICROPHONICS

It has been shown[5] that a slight increase in the potassium chloride concentration in the perilymph reversibly abolishes both the neural and the microphonic responses of the cochlea. It seemed that KCl acted more readily when introduced into scala tympani

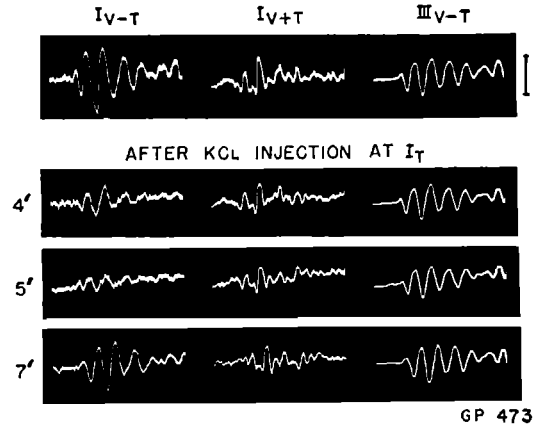

FIG. 7. Effect of KCl-rich Ringer solution introduced into scala tympani of the basal turn upon the microphonic response of the basal turn (left column), the neural responses (middle column) and the microphonic response of the third turn (right column). The three channels were photographed simultaneously. Sound stimuli were 500-cps tone pips. The bar represents 100 μv for the left and middle columns and 316 μv for the right column. Minutes after injection are indicated at the left.

[13] H. Davis, Advancement of Sci. 9, 420–424 (1953).
[14] H. Davis, Med. Bull. St. Louis Univ. 5, 43–48 (1953).
[15] H. Davis, *Transactions of the Fourth Conference of Josiah Macy, Jr., Foundation on Nerve Impulse* (Josiah Macy, Jr., Foundation Publications, Packanack Lake, New Jersey, 1953), pp. 138–139.

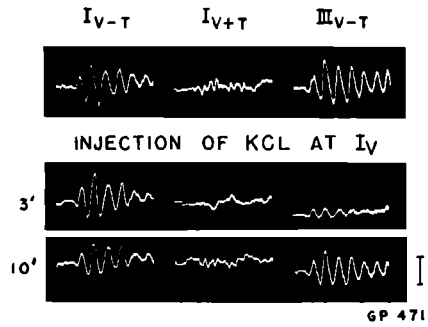

FIG. 8. Similar to Fig. 7 except that the KCl-Rich ringer solution was introduced in scala vestibuli of the basal turn and allowed to flow out of scala tympani of the third turn. The bar, 100μv for the left and middle columns and 316 μv for the right column. (Neural responses are present after three minutes but are atypical. The microphonic is reduced in the third turn but *not* in the basal turn.)

than when injected into scala vestibuli. This impression has now been confirmed.

Microphonic responses to 500-cps tone pips were recorded simultaneously from both the third and the basal turns with two pairs of nichrome-steel wire electrodes (our usual vestibuli-to-tympani differential recording). As we already know[4-6] these pips elicit microphonic responses from the first, second and third turns. In the experiment of Fig. 7, a pair of small holes for perfusion of the cochlea, each approximately 100μ in diameter, were made in the scala tympani of the basal turn, one apical and the other basal to the recording electrode in this scala. A mixture, stained lightly with neutral red, of one part of an isotonic KCl solution with three parts of mammalian ringer solution was introduced, through a glass pipette, into scala tympani in the basal turn. Outflow of the KCl solution from the other hole in scala tympani of the basal turn was observed. After this perfusion the accumulated fluid in the bulla was removed with the glass pipette and small pledgets of cotton and the microphonic responses were recorded as before.

This procedure caused a transient but unmistakable reduction in the microphonic response from the basal turn about 4 to 5 minutes after the injection. Meanwhile there was only a very slight reduction in the response from the third turn, indicating that the KCl solution did not spread significantly to the third turn, and therefore certainly not to the scala vestibuli of the basal turn. Later, due presumably to dilution of KCl by the perilymph and by spread of some KCl along the scala tympani, the response from the basal turn recovered almost completely.

In the converse experiment (Fig. 8) the recording electrodes, two pairs of nichrome-steel wire electrodes, were placed as before in the third turn and the basal turn. The holes for perfusion, however, were made with one near the basal end of the scala vestibuli and the other in the scala tympani of the third turn (on the basal side of the recording electrode there). The KCl solution introduced into the scala vestibuli of the basal turn therefore passed up the vestibuli scala to the helicotrema and on down the scala tympani to the third turn, but the scala tympani of the basal turn was, at least at the time of perfusion, free from KCl. At the moment when the microphonic response of the third turn had almost disappeared, the response of the basal turn did not show any observable change in its amplitude.

In both experiments the KCl solution acted only in that part of the cochlea where it replaced the fluid in the scala tympani. This is more strong evidence that the microphonic responses are generated by the cells on the tympanic side of the scala media. Also, *the basilar membrane is apparently permeable to potassium ions* but the space filled with endolymph does not let KCl pass through freely.

In Figs. 7 and 8 the middle columns show the neural responses to the 500-cps pips. These nerve action potentials are reduced by injection of the KCl solution into any part of the cochlea but they are not completely abolished unless almost the whole cochlea is treated with KCl simultaneously. This confirms our previous conclusion[5,6] that low-frequency sounds excite the whole cochlea, both the upper and lower turns.

8. THE ENDOLYMPHATIC SPACE

According to Smith, Lowry, and Wu,[11,15,16] the concentration of potassium in the endolymph is far higher than in the perilymph, almost 25 times as high. We found, while altering the hydrostatic pressure in scala media (see Sec. 4), that isotonic potassium chloride solution is well tolerated in scala media but that ringer solution is not. This is a clear, although qualitative, confirmation of the findings of Smith, Wu, and Lowry.

The high potassium content of endolymph shows that the walls of the endolymphatic space must be almost impermeable to potassium ions. All of these facts force us to modify our concept of the extent of the "endolymphatic" and "perilymphatic" spaces. *The "endolymphatic space" must be bounded by the limbus, Reissner's membrane, the stria vascularis, the outer and inner sulcus cells, the cells of Hensen and Claudius and the reticular lamina,—and not by the basilar membrane.* This wall is shown by the heavy line in Fig. 3. The basilar membrane seems to be permeable to KCl. The nerve fibers and the hair cells in the organ of Corti must be immersed, not in endolymph (which contains more than enough potassium to abolish neutral and microphonic responses completely), but in perilymph. The ionic composition of perilymph is known to be like that of other body fluids.[11,15,16]

According to Katsuki and Covell,[17] the reticular

[16] Smith, Lowry, and Wu, Laryngoscope **64**, 141-153 (1954).
[17] Y. Katsuki and W. P. Covell, Laryngoscope **63**, 1-17 (1953).

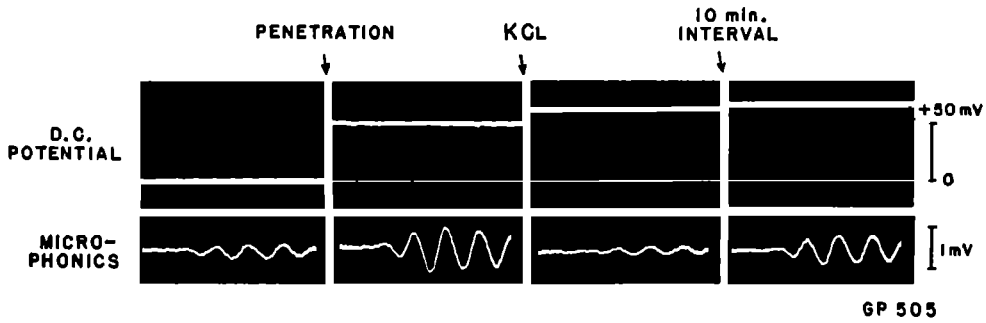

FIG. 9. Effect of isotonic KCl solution on the dc potential (top) and the microphonic response (bottom) of the scala. Sound stimuli were 500-cps tone pips. The KCl was injected (see text) between the second and third exposures.

lamina on the upper surface of the organ of Corti is a solid continuous layer of considerable mechanical strength and thickness except at the spots where the tops of the hair cells penetrate it. Apparently this membrane and the cells of Claudius and of Hensen constitute the main diffusion-barrier between the scala media and the scala tympani.

9. LACK OF EFFECT OF KCl ON THE DC POTENTIAL

In contrast to the marked effect on the microphonics of increased potassium in scala tympani, the dc endolymphatic potential is scarcely affected at all. In the experiment of Fig. 9, the microphonic response was recorded between a nichrome-steel wire electrode inserted in scala tympani of third turn and a microelectrode pushed into scala media of the same turn. The dc potential difference was measured between the microelectrode in scala media and a large glass-pipette electrode in scala tympani of the basal turn. Two holes were made for injection of an isotonic KCl solution, one into scala tympani of the basal turn and the other at the apex. (In some other experiments the second large hole for perfusion was in the scala vestibuli of the basal turn.) The records at the extreme left (in Fig. 9) were taken when the microelectrode was still in the spiral ligament. The amplitude of the microphonic response was small and there was practically no dc potential at the tip of the microelectrode. When the microelectrode penetrated the stria vascularis and reached the endolymph, the microphonic response increased and the large positive dc potential appeared as usual. Then isotonic KCl solution was introduced into the scala tympani. The amplitude of the microphonic response promptly decreased, but at this moment there was still a large dc potential in scala media.

The positive dc potential evidently does not depend on the concentration ratio of potassium inside to potassium outside of the scala media. In this respect it differs fundamentally from the intracellular potential. The latter, in nerve and muscle cells, is closely and quantitatively related to the ratio of potassium concentrations inside and outside the cell.[18] The clearest demonstration that we are dealing with something more than differences in potassium concentrations is the existence of a dc potential difference of about 120 mv between the endolymph and the interior of a hair cell while the concentrations of potassium in the endolymph and in protoplasm are approximately the same. The nature of the special metabolic activity that produces extracellular potential differences and does it without differences in potassium concentration is entirely unknown. Our best present evidence seems to locate this activity in the hair cells. However, the differential suppression of the microphonic by KCl without altering the dc endolymphatic potential implies that, even though they may be located in the same cell, the mechanism of the microphonic, i.e., the *modulation* of the dc, is to some extent independent of the mechanism of *generation* of the dc endolymphatic potential.

[18] See A. L. Hodgkin and A. F. Huxley, Cold Spring Harbor Symposia Quant. Biol. **17**, 43–52 (1952).